Improved spatial learning performance of fat-1 mice is associated with enhanced neurogenesis and neuritogenesis by docosahexaenoic acid

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Docosahexaenoic acid (DHA), an n-3 long chain polyunsaturated fatty acid (LC-PUFA), highly enriched in the central nervous system, is critical for brain development and function. It has been shown that DHA deficiency impairs cognitive performance whereas DHA supplementation improves the condition. However, the mechanisms underlying the role of DHA in brain development and function remain to be elucidated. By using transgenic fat-1 mice rich in endogenous n-3 PUFA, we show that increased brain DHA significantly enhances hippocampal neurogenesis shown by an increased number of proliferating neurons and neuritogenesis, evidenced by increased density of dendritic spines of CA1 pyramidal neurons in the hippocampus. Concurrently, fat-1 mice exhibit a better spatial learning performance in the Morris water maze compared with control WT littermates. In vitro experiments further demonstrate that DHA promotes differentiation and neurite outgrowth of neuronal cells derived from mouse ES cells and increases the proliferation of cells undergoing differentiation into neuronal lineages from the ES cells. These results together provide direct evidence for a promoting effect of DHA on neurogenesis and neuritogenesis and suggest that this effect may be a mechanism underlying its beneficial effect on behavioral performance.

cognitive function | omega-3 fatty acids | transgenic fat-1 mice | neural stem cells

Docosahexaenoic acid (22:6n-3, DHA) is an omega-3 (n-3) long chain polyunsaturated fatty acid (LC-PUFA) that is highly enriched in the central nervous system, particularly in the synaptosomal membrane, synaptic vesicles, growth cones, and the retina photoreceptors (1). DHA serves not only as a building block for neural development, but also as a functional molecule to maintain proper fluidity of neuronal membranes and modulate neurochemical, gene expression, and memory processes (2). Clinical evidence indicates that term infants given formula supplemented with either DHA plus arachidonic acid or DHA alone have substantially better visual acuity (3) and improved cognitive ability (4). Animal studies revealed that deficiency of DHA resulted in a poorer performance on cognitive and behavioral tests whereas supplementation with DHA led to recovery of learning and memory-related performance (5). Furthermore, neurodegeneration and neuropsychiatric disorders are related to a low level of DHA, and supplementation with DHA ameliorated the symptoms (6). These studies suggest that DHA plays a key role in the development and function of the central nervous system. However, the mechanisms underlying the effects of DHA on brain development and function remain to be elucidated.

The dentate gyrus (DG) has been an intensively investigated area of the hippocampus because DG is one of the few brain regions where neurogenesis takes place throughout the life span of mammals and where new memories are formed (7). In addition, DG is critical for normal cognitive function, and dysfunctions in this region are linked to diverse clinical conditions (e.g., depression, epilepsy, and traumatic brain injury). It has been shown that the state of neurogenesis and density of dendritic spines in the hippocampus are closely associated with memory formation. Whether an effect on the neurogenesis and synaptic structures in the hippocampus contributes to the role of DHA in brain development and function warrants further investigation.

For studies evaluating the effects of DHA in vivo, dietary supplementation with n-3 fatty acids (usually from fish or algal oils) is a conventional approach for enriching tissues with DHA. However, feeding animals with different experimental diets may impose confounding effects on the results because of many variables arising from the diets and feeding procedures. Thus, an appropriate animal model that can eliminate confounding factors of diet would be very helpful for such evaluation. The fat-1 mouse is a transgenic model rich in endogenous n-3 fatty acids, particularly DHA (8). Fat-1 transgenic mice express the *C. elegans fat-1* gene and are capable of producing n-3 fatty acids from the omega-6 type, leading to an abundance of n-3 fatty acids in their tissues and organs without the need of a dietary n-3 fatty acid supply. This model allows well-controlled studies to be performed without the interference of potential confounding factors of diet, and thus is a tool for the evaluation of DHA effects. In the present study, we used the fat-1 mouse model, in combination with in vitro experiments, to investigate the effects of DHA on neurogenesis and neuritogenesis, as well as their relation to behavioral performance.

Results

Brain Fatty Acid Profile of Fat-1 and WT Littermates. The LC-PUFAs with 22 carbons in length are major and important lipid molecules in the brain. The total amount of VLC-PUFA (sum of n-6 and n-3) in the hippocampus is about the same in WT (16.8%) and fat-1 mice (17.0%). But the composition of these fatty acids is quite different in WT and fat-1 mice (Fig. 1). As a result of the conversion of n-6 to n-3 in the fat-1 mice, the level of n-3 LC-PUFA, DHA (22:6n-3) and docosapentaenoic acid, DPA, (22:5n-3), are significantly higher in fat-1 (13.8% and 0.14%, respectively) than in WT mice (9.6% and 0.02%, respectively), whereas the n-6 LC-PUFA, DPA (22:5n-6) and docosatetraenoic acid, DTA (22:4n-6), are remarkably lower in fat-1 (0.6% and 2.5%, respectively) than in WT mice (4.3% and 2.9%, respectively). The resulting ratios of n-6/n-3 LC-PUFA in WT and fat-1 mice are 0.83 and 0.23, respectively. There is no significant difference in the level of fatty acids in WT and fat-1 mice.

Hippocampal Neurogenesis Is Enhanced in Adult Fat-1 Transgenic Mice. To examine whether cell proliferation in the DG area of the hippocampus is enhanced in fat-1 mice with high levels of DHA, BrdU was used to label the newly generated cells by immunohistochemisty staining with a specific antibody against BrdU. BrdU-positive cells, generally in pairs or clusters, were distributed at the inner border of the GCL and hilus, similar to that reported in ref.

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The authors declare no conflict of interest.

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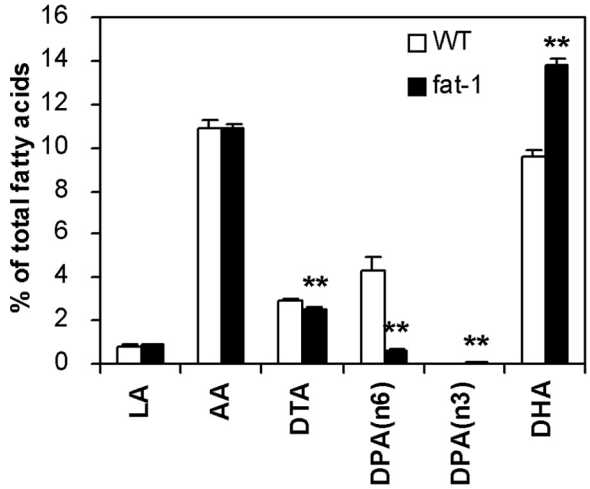

Fig. 1. Fatty acid composition in the hippocampus of WT and fat-1 transgenic mice. Fatty acids were extracted, methylated, and analyzed by gas chromatography. Linoleic acid (LA) and arachidonic acid (AA) remain the same level between WT and fat-1 mice. DHA and docosapentaenoic acid (DPA, n-3) increase whereas docosatrienoic acid (DTA, n-6) and DPA(n-6) decrease significantly in fat-1 mice. Results are means ± SEM. **, $P < 0.01$; $n = 3$, Student's t test.

9. As shown in Fig. 2, the number of BrdU-positive cells in SGZ and GCL of the DG area is greater in fat-1 mice than in WT mice (WT: 2042 ± 108; fat-1: 3110 ± 580; $P < 0.05$), suggesting that DHA potentiates hippocampal cell proliferation in mice.

Dendritic Spine Density Is Increased in the Hippocampus of Adult Fat-1 Transgenic Mice. To examine whether neuritogenesis in the hippocampus is different in fat-1 and WT mice the dendritic spine density of the hippocampus was measured by Golgi-Cox staining. As shown in Fig. 3, the spine density in CA1 pyramidal neurons is significantly higher in fat-1 mice than in their WT counterparts (WT, 9.3 ± 0.8/10 μm; fat-1, 13.1 ± 1.0/10 μm; $P < 0.05$), suggesting that DHA promotes neuritogenesis in the mouse hippocampus. We further examined whether the expression of presynaptic and postsynaptic genes was up-regulated accordingly. Measurement of gene expression by quantitative real time PCR showed that F-actin, GAP43, GluR1, PSD95, and synapsin-1 were up-regulated by 140%, 218%, 171%, 249%, and 160%, respectively, in the hippocampus of adult fat-1 transgenic mice compared with WT mice (Fig. S1).

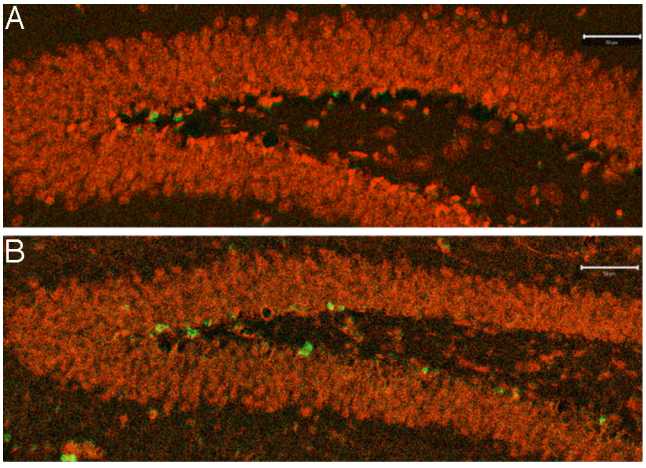

Fig. 2. Hippocampal neurogenesis is promoted in adult fat-1 mice. More BrdU+ (green) cells were found in dentate gyrus (DG) of fat-1 mice (*B*) than that of WT mice (*A*) as shown by confocal microscope. (Scale bar, 50 μm.)

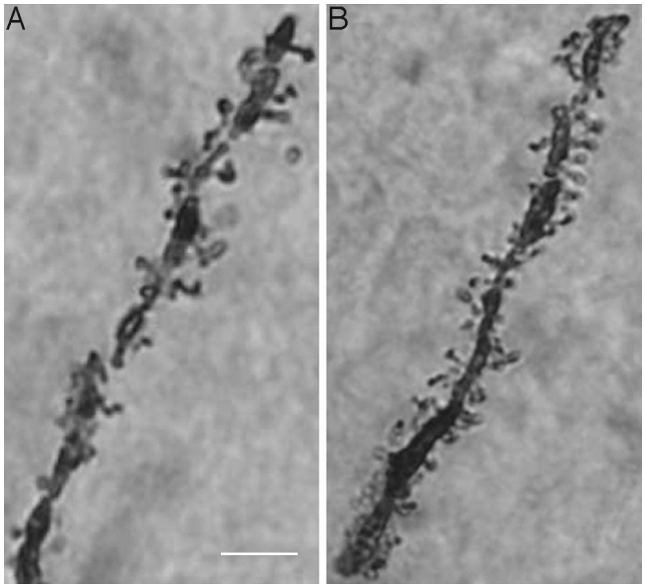

Fig. 3. Density of dendritic spines is increased in adult fat-1 hippocampus. Golgi-Cox stained dendritic spines from WT (*A*) and fat-1 (*B*) mice. Note the density of dendritic spines along dendrite. (Scale bar, 5 μm.)

DHA Enhances Neurite Outgrowth of Neural Cells Differentiated from Embryonic Stem Cells in Vitro. To validate the effect of DHA on neuritogenesis in vitro, we induced differentiation of G-olig2 embryonic stem cells into neuronal cells in defined serum-free medium (10) with or without DHA. Treatment of cells with DHA significantly increased cellular DHA content (Fig. S2). The neuritogenic activity of cells undergoing neuronal differentiation from embryonic stem cell was significantly promoted by DHA, as shown by increased neurite length, neurite number and number of neurite branches (Fig. 4, $P < 0.01$). The number of neurite branches was the most significantly different (2.85-fold in DHA-treated cells, $P < 0.01$). The neuritogenic index in DHA-treated cells was increased by 174% compared with the control ($P < 0.01$). Five micromolar DHA also enhanced neurite outgrowth from embryoid bodies (EB) undergoing differentiation (Fig. 4*B*), suggesting DHA promotes neuritogenesis of cells differentiated from embryonic stem cells in vitro.

DHA Promotes Neural Differentiation and Proliferation of Neural Cells in Vitro. To examine the effect of DHA on neural differentiation from embryonic stem cells, dispersed cells from ES were induced to differentiate with or without 5 μM DHA. Some of the cells were immunostained with neuron marker Tuj1 (Fig. 5). The number of Tuj1-positive cells was increased by 207% compared with the control (control, 4.6 ± 0.7%; DHA, 9.5 ± 1.0%; $P < 0.01$). Furthermore, neurons (Tuj1+) in the DHA-treated group exhibited more and longer neurite outgrowth, indicating DHA potentiates differentiation of ES cells into neurons in vitro.

Labeling with BrdU was used to determine the proliferation of cells in vitro. BrdU was added to the culture medium during the differentiation period. BrdU incorporation was determined by immunocytochemistry staining. The number of BrdU-positive cells in the DHA-treated group increased significantly by 137% compared with the control (Fig. S3; control, 51.6 ± 6.7%; DHA, 70.5 ± 2.2%; $P < 0.01$), indicating that DHA stimulates the proliferation of cells undergoing differentiation from embryonic stem cell in vitro, which is consistent with the in vivo observations.

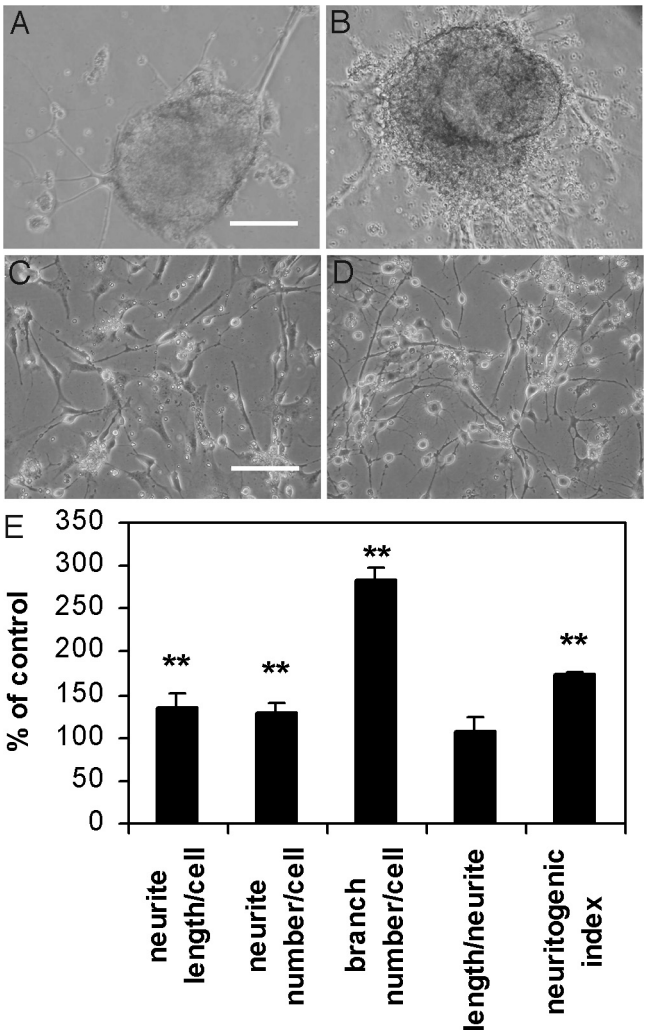

Fig. 4. DHA enhances the neurite outgrowth of neuronal cells differentiated from G-olig2 embryonic stem (ES) cells. The ES cells were induced to differentiate into neuronal cells in defined serum-free medium for 13 days. (*A–D*) Phase contrast microscope. Embryoid bodies (EBs) (*B*) and singles cells (*D*) treated with 5 μM DHA have more and longer neurites compared with the control EBs (*A*) and single cells (*C*). (*E*) Neurite analysis of length and number of neurites per cell, branch number per cell, length per neurite, and neuritogenic index (neurite number $\times$ neurite length) from *C* and *D*. Results are means $\pm$ SD (3–5 experiments). **, $P < 0.01$ compared with the control (Student's *t* test). (Scale bar for *A* and *B*, 100 μm; Scale bar for *C* and *D*, 20 μm.)

Spatial Learning Assessment. The Morris water maze was used to assess the mouse's spatial learning performance. Fat-1 mice spent less time finding the hidden platform underneath the water than their WT counterparts ($F_{1,11} = 7.992$, $P = 0.016$) during the acquisition and training phase as illustrated by the latency value in the test with the hidden platform (Fig. 6), indicating that the spatial learning ability of fat-1 mice was better than WT mice. However, there was no difference regarding the retention time in the probe trial between WT and fat-1 mice ($P > 0.05$; Fig. S4). In the visible platform trail, no difference in the escape latency was observed between the WT and fat-1 mice ($P > 0.05$; Fig. S5). This can exclude the possibility that the observed difference in spatial learning is due to motor or sensory factors.

Discussion

In the present study, we used the fat-1 mouse model, in combination with in vitro experiments, to investigate the effects of DHA on neurogenesis and neuritogenesis, as well as their relation to behavioral performance. Our results demonstrate that increased brain DHA significantly enhances hippocampal neurogenesis and neuritogenesis, accompanied with a better spatial learning performance. Thus, this study provides evidence that increased neurogenesis and neuritogenesis in the hippocampus may provide a basis for the beneficial effect of DHA on behavioral performance.

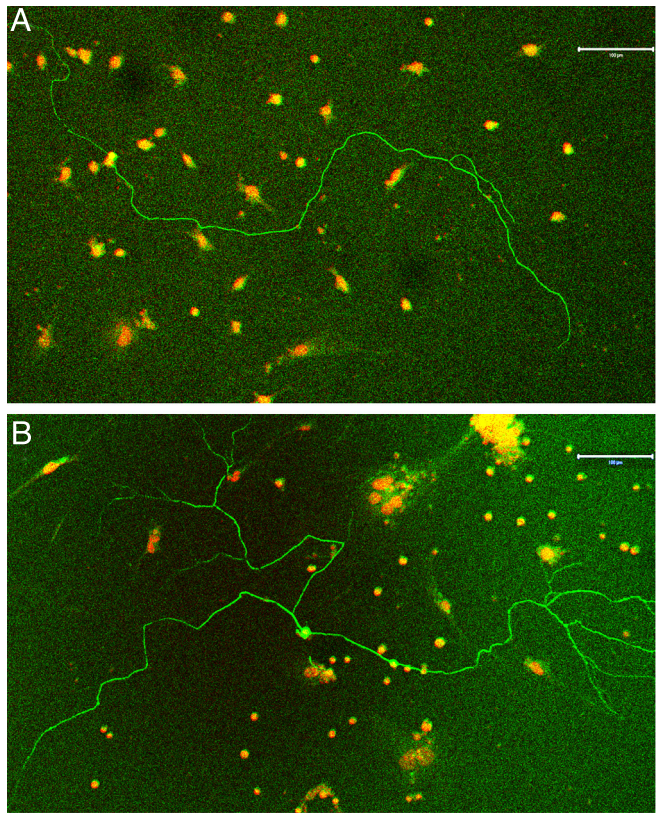

Fig. 5. DHA (5 μM) supplementation increases the number of Tuj1+ cells after differentiation in defined serum-free medium for 13 days from G-Olig2 ES cells. Confocal images of Tuj1 immunostaining in control (*A*) and DHA (*B*) groups, Tuj1 (green), PI (red). (Scale bar, 100 μm.)

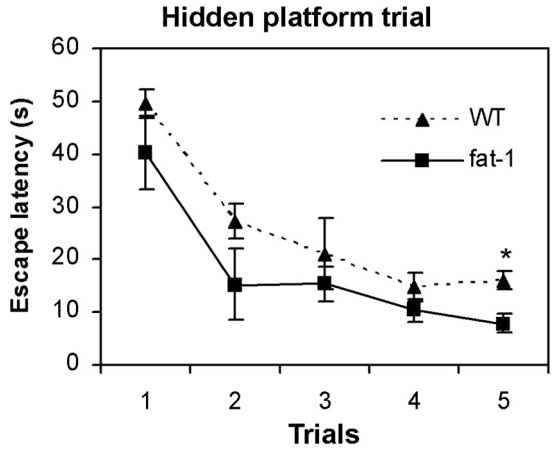

Fig. 6. Performance in the Morris water maze is improved in fat-1 transgenic mice. The latency per testing session represents the average of 4 trials of all animals in each group. Results are means $\pm$ SEM. In hidden platform trial, the escape latency (s) of fat-1 mice ($n = 6$) is significantly lower than that of WT mice ($n = 7$); *, $P = 0.016$ ($F_{1,11} = 7.992$; two-way ANOVA followed by post-hoc Tukey test).

The unique aspect of the present study is the use of the fat-1 transgenic mouse model instead of dietary supplementation to create the difference of DHA content in brain tissues. Two groups of animals (WT and fat-1) could be fed with the same diet, but their tissue fatty acid composition would be quite distinct, with no change in the mass of total fatty acids. Under the dietary regime used, the nontransgenic (WT) mice exhibit a typical profile of partial n-3 fatty acid deficiency in the brain, characterized by a low level of DHA (9.6%) and a high level of n-6 DPA (22:5n-6) (4.3%). It is well recognized that when the brain is deficient in DHA, the n-6 counterpart DPA is reciprocally increased (11). However, it is also known that the n-6 DPA (22:5n-6) cannot functionally substitute for DHA in the brain (12). In the fat-1 transgenic mice, the n-6 DPA (22:5n-6) can be converted to DHA (22:6n-3), leading to a higher level of DHA (13.8%) and a much lower level of n-6 DPA (0.6%). Interestingly, other than the 22-carbon fatty acids [DHA (22:6n-3), n-3 DPA (22:5n-3), n-6 DPA (22:5n-6), and DTA (22:4n-6)], there is no significant difference in other fatty acids in the brain. Given the fact that the 22-carbon fatty acids are especially abundant in the central nervous system and might be structurally and functionally important for it, the fat-1 mouse model with a significant difference in these fatty acids in the brain and, more importantly, free of confounding factors because of different diets, make it a desirable tool for the evaluation of the functional importance of these fatty acids, particularly DHA, in the brain.

With the fat-1 mouse model, we were able to show an effect of brain DHA content on both neurogenesis and neuritogenesis. Evaluation of hippocampal adult neurogenesis by BrdU incorporation assay indicated that the number of BrdU-positive dividing cells was significantly higher in the hippocampus of fat-1 transgenic mice than that of WT littermates (Fig. 2). This observation is consistent with the findings of recent studies in rats fed with DHA (13) and in lobsters supplemented with alpha linolenic acid and eicosapentaenoic acid (14). In addition, our observation agrees with the finding that corneal nerve regeneration is induced by DHA (15), particularly when combined with nerve growth factor. Examination of neuritogenesis by Golgi-Cox staining for dendritic spine density of the hippocampus revealed that the spine density in CA1 pyramidal neurons was markedly higher in fat-1 mice than in WT counterparts (Fig. 3). This is supported by the increased expression of the dendritic spine related genes F-actin, GAP43, GluR1, PSD95, and synapsin-1 (Fig. S1) in the hippocampus of adult fat-1 transgenic mice. This positive effect on dendritic spine density is consistent with a recent finding in the adult gerbil showing that supplementation with DHA increases the number of dendritic spines in the hippocampus (16). Thus, our results demonstrate that animals with higher levels of brain DHA have increased hippocampal neurogenesis and neuritogenesis, suggesting a role for DHA in promoting these processes.

Further evidence for the promoting effect of DHA on neurogenesis and neuritogenesis came from our in vitro experiments examining the effects of DHA on differentiation and proliferation of mouse embryonic stem cells, and on the neurite outgrowth of differentiated neural cells (Figs. 4 and 5 and Fig. S3). Our results clearly show that DHA not only promotes differentiation of embryonic stem cell into neurons (Fig. 5), but also stimulates the proliferation of cells undergoing differentiation (Fig. S3). In addition, DHA administration significantly increases the number and length of neurites, particularly the number of neurite branches on cells undergoing differentiation (Fig. 4). These results are consistent with the observations showing that DHA enhances the differentiation of neural stem cells into mature neuronal cells (13, 17–19). These data together support the notion that DHA is a key element for neurogenesis and neuritogenesis.

It is well-established that the levels of learning and memory is associated with the levels of neurogenesis and the number and complexity of dendritic spines on neuronal cells (20, 21). In this context, it's expected that fat-1 mice with enhanced neurogenesis and neuritogenesis should have a better spatial learning performance. Indeed, our behavioral tests showed (Fig. 6) that fat-1 mice spent less time (escape latency) than WT mice finding the hidden platform during the training of the Morris water maze test, indicating a better spatial learning ability in fat-1 animals with higher levels of DHA in the brain. This beneficial effect of DHA on spatial learning is also supported by previous observations (22, 23). Our findings suggest that the enhanced neurogenesis and neuritogenesis by DHA may be an important mechanism underlying its beneficial effect on behavioral performance.

The molecular mechanisms underlying the promoting effect of DHA on neurogenesis and neuritogenesis remain to be elucidated. DHA can influence cell function through multiple mechanisms ranging from modulation of membrane properties to regulation of signal transduction and gene expression. DHA is a key building block for membrane synthesis and is important for neuronal growth and repair. DHA esterified into phospholipids of the plasma membrane bilayer significantly alters many basic membrane properties, including fluidity, flexibility, permeability, electrostatic behavior, and/or direct interaction with membrane proteins, and consequently regulates the neurotransmission, signal transduction, and formation of lipid rafts. However, the unesterified free DHA (released from phospholipids catalyzed by phospholipase A2) exerts complex changes in gene expression in the brain by its interactions with transcriptional factors and binding to specific regions of DNA (24, 25). DHA is a ligand for RXR (26) and PPAR (27) through which expression of genes involved in neurogenesis and neuritogenesis may be regulated at the transcriptional level. Recent studies (28, 29) revealed that DHA as well as other omega-3 fatty acids broadly regulated the expression of genes ranging from energy metabolism, signal transduction, cell adhesion, apoptosis, and synaptic activities to neurogenesis and maturation. Furthermore, a recent study identified syntaxin 3 (STX3) as a mediator of the action of n-3 and n-6 fatty acids in membrane expansion at the growth cone (30), an initial process of neurite outgrowth. In addition, DHA can be further metabolized to neuroprotective mediators, specifically neuroprotectin D1, that exhibit protective and anti-inflammatory activities in the brain as well as in other tissues (31–33). Therefore, it's likely that DHA exerts its bioactivity through multiple pathways.

In summary, the present study looks at the effect of DHA on both the structural and functional aspects of the brain using a model to exclude dietary confounding factors, and demonstrate that increased brain DHA enhances neurogenesis and neuritogenesis in the hippocampus and thereby improves spatial learning performance. The results of this study not only provide insight into how DHA can influence behavioral performance but also suggest a role for DHA in prevention and treatment of nerve injury and neurodegenerative diseases.

Materials and Methods

Animals. Transgenic fat-1 mice were created and bred as described in ref. 8. The mice were backcrossed onto a C57BL/6 background. Animals were fed a diet (Mod AIN-76A with safflower oil; Land O'Lakes Purina) with a fatty acid profile (Table S1) high in n-6 and very low in n-3 fatty acids, until the desired age (10–12 weeks) for experiments. Heterozygous fat-1 male mice exhibiting significant phenotype and WT littermates were subject to the experiments.

Lipid Analysis. The fatty acid composition of total cellular lipids was analyzed as described in ref. 34. Briefly, fatty acids were extracted from cells (2×10^6) or brain tissues and methylated by adding 1.5 mL hexane and 1.5 mL 14% BF3/MeOH reagent (Sigma–Aldrich), and then heating at 100 °C for 1 h after blanketed the tube with nitrogen. The upper hexane layer was removed and concentrated under nitrogen. Fatty acid methyl esters were analyzed by gas chromatography using a fully automated 6890N Network Gas Chromatographs System (Agilent Technologies) equipped with a flame-ionization detector.

Proliferation Assay in Vivo by BrdU Staining. Mice received one daily i.p. injection of 50 mg/kg BrdU (Sigma–Aldrich) dissolved in PBS for 5 days. Forty-eight hours

after the last injection, the mice were killed by cervical dislocation, brains were quickly removed and fixed in 4% paraformaldehyde for 24 h. Coronal sections (10 μm) were cut on a microtome and processed for the immunohistochemical staining with anti-BrdU monoclonal antibody (Lab Vision) and secondary anti-Alexa Fluor 488 antibody (Invitrogen). BrdU positive cells were counted on every third section throughout the entire rostrocaudal extension of the subgranular zone (SGZ) and granule cell layer (GCL) of DG in the hippocampus. The sum was multiplied by 3 to estimate the total number of BrdU-positive cells in each hemisphere.

Golgi-Cox Staining. Mice were killed by cervical dislocation, brains were quickly removed and placed in 10 mL Golgi-Cox solution (35) for 22 days. Then the brains were placed in 30% sucrose solution for 3 days, cut on a microtome at 100 μm. Slices were counterstained with methylene blue to distinguish anatomical landmarks. The analyzers were blind to the experimental group. Ten dendrites of the same size in diameter from CA1 pyramidal neurons were randomly selected from 5 slices for individual animal. Protrusion of <5 μm in length was defined as a spine. Spines were counted under a 100$\times$ oil-immersion objective using an image-combining computer microscopy program MDS120 microscopy documentation software (Kodak). Spine density was calculated by dividing the total number of spines per dendrite by the total length of the dendrite.

Quantitative Real-Time PCR. Total RNAs from the hippocampus of WT and fat-1 mice were extracted by using Qiagen RNeasy kit and converted into first strand of cDNAs by Promega reverse transcription kit according to the manufacturer's instructions. PCR was performed on the ABI PRISM 7000 Sequencing Detection System (Applied Biosystems). SYBR Green Master Mix (ABgene) with ROX as the reference dye was used for the PCR step according to the manufacturer's instructions. Normalized cycle threshold (ΔCt) was calculated by subtracting Ct value of GAPDH from Ct value of target genes. Relative gene expression level of fat-1 mice compared with WT mice was determined by the formula: $2^{\Delta Ct(wt) - \Delta Ct(fat\text{-}1)}$.

ES Cell Culture and Neuronal Lineage Differentiation. Undifferentiated G-Olig2 ES cells (ATCC) were cultured on mytomycin C-treated mouse embryonic fibroblasts STO feeder cells (ATCC) in ES-complete-medium as described in ref. 36. Embryoid bodies (EBs) were formed by culturing the ES cells in 10-cm ultra-low attachment dishes (Corning) at 1×10^6 per dish in complete medium without LIF. The medium was changed every other day. The EBs were spontaneously formed, and collected after 4 days of culturing for the following experiments. Neural lineage differentiation was induced as described (36, 37). The EBs were trypsinized to single cells with 0.25% trypsin and 1 mM EDTA. The single cell suspension and EBs were cultured in polyornithine coated tissue culture plates in ITS medium (1:1 DMEM/F12 with the addition of 5 μg/mL insulin, 50 μg/mL transferrin, and 30 nM sodium selenite) (Invitrogen) with or without 5 μM DHA (Sigma–Aldrich). The medium was changed every other day. After 6 days, ITS medium was replaced by N2 medium (Invitrogen) with or without 5 μM DHA. The differentiation into neurons was induced for 7 days in N2 medium.

Analysis of Neurite Formation. The cells were digitally photographed from randomly selected image fields (total magnification 200$\times$). The neurite length, number, and branch number were analyzed using the Image-J program (National Institutes of Health, Bethesda, MD). Only those primary neurites originating directly from soma that were longer than the diameter of cell body were measured in a double blind study (38, 39). The length and number of neurite per cell, branch number per cell, length per neurite and neuritogenic index (neurite number $\times$ neurite length) were calculated.

Immunocytology. Cells were fixed in 4% paraformadehyde in PBS for 30 min, and permeabilized in 1% Triton X-100 (for Tuj1 staining) or in 0.1% digitonin (for BrdU staining) for 15 min. The first antibody against neuronal class III ß-tubulin (Chemicon) or BrdU (Lab Vision) was incubated with cells overnight at 4 °C. After washing 3 times (5 min each) with PBS, cells were incubated 1 h with secondary anti-Alexa Fluor 488 antibody (Invitrogen). The Tuj1 or BrdU-positive cells were counted under fluorescent microscope.

Spatial Learning Test. Morris water maze test was used to assess the spatial learning that mainly involves the hippocampus (40). For the hidden platform trail, the mice were placed randomly in the water facing the wall in each quadrant to search the platform for 60 s. Mice failed to reach the platform within the time-period were gently guided to the platform and allowed to stay for 10 s, and an escape latency time of 60 s was recorded. Each mouse was given 2 trials each day. The time spent to reach the platform (escape latency time) was recorded for each trial. In the last day of experiment, a probe trial was performed by removing the platform from the tank and allowing animals to search for the platform for 60 s. The time the mice spent in the target quadrant was recorded. To exclude the effect of motor and sensory factors on maze performance, the mice were subject to a visible platform trial similar to the hidden platform trail except that the escape platform was clearly marked and elevated 1 cm above the water.

Statistics. Comparisons were made between 2 treatments or 2 genotypes by the Student's *t* test. Difference of escape latency time of hidden platform trails between WT and fat-1 mice was evaluated by two-way ANOVA analysis followed by post hoc Tukey test. Differences were considered significant at the level of $P < 0.05$.

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